REMARKS

This Request for Continued Examination and Response to Office Action is filed in connection with the Office Action mailed December 20, 2005. Claims 31, 34 to 36, 38, 40, 43, 47 to 49, 51, 52, 54, 55, 71 to 73, 76, 78 to 80, 82, 83 and 85 to 113 are pending. Claims 89 to 113 stand withdrawn from consideration as directed to non-elected subject matter. Claims 48, 52, 79, 83 and 89 to 113 have been cancelled herein without prejudice. Applicants maintain the right to prosecute the cancelled claims in any related application claiming the benefit of priority of the subject application. New claims 114 to 120, which depend from elected claims 31 and 71, have been added. Accordingly, upon entry of the Response, claims 31, 34 to 36, 38, 40, 43, 47, 49, 51, 54, 55, 71 to 73, 76, 78, 80, 82, 85 to 88 and 114 to 120 are under consideration.

Applicants wish to thank the Examiner and their supervisor for the Interview held February 8, 2006, during which all grounds for rejection were discussed. The amendments, Declaration and Exhibits submitted herewith are consistent with the substance of the Interview and are believed to address all outstanding grounds for rejection.

Regarding the Claim Amendments

The amendments to the claims are supported throughout the specification or were made to address informalities. In particular, the amendments to claims 31 to 71 reciting "transforming gut mucosal stem cells, pluripotent or multipotent progenitor cells, or gastrointestinal mucosal stem cells, pluripotent or multipotent progenitor cells....thereby producing transformed mucosal tissue cells " is supported, for example, by claims 1 and 31, as originally filed; at page 9, lines 24-28, which discloses that the invention "includes methods of targeting expression of any protein of interest to endocrine cells in the gastrointestinal tract of a subject such that the protein is released into the bloodstream of the subject in a regulated manner;" at page 10, lines 1-3, which discloses that "when the gene construct is incorporated into endocrine cells, the encoded protein will be expressed and secreted in a regulated manner;" at page 10, lines 17-18, and lines 23-25, which discloses that "there are provided methods of generating a mucosal cell that produces a protein regulatable by a nutrient," and that in one embodiment "the mucosal cell is contacted with the polynucleotide *in vivo*;" at page 10, lines 27-29, which discloses that "the mucosal cell is a stem cell or a pluripotent or multipotent progenitor cell" (see, also, page 25, lines 3-6 and 24-25); at page 37, lines 19-23, which discloses *in vivo* delivery to produce

encoded protein in the subject; and at page 39, lines 19-22, which discloses administration by routes including, for example, intra-cavity. The amendment to recite "orally" contacting said transformed mucosal tissue endocrine cells in the subject with an amount of sugar, polypeptide, amino acid or fat, is supported, for example, at page 16, lines 1-3, which discloses that a nutrient means "ingestible or consumable material such as that present in food or drink" that "include sugars....fats....polypeptides, amino acids;" at page 18, lines 11-15, which discloses "chemicals or drugs that are orally active, but not normally found in food," which "when consumed, stimulates expression of a nucleic acid;" and at page 48, lines 18-21, which discloses that "oral nutrient challenges promptly stimulated the release of human insulin from K cells," which "confirmed that insulin secretion from gut K cells is indeed meal-regulated." The amendment reciting "transcription or secretion" is supported, for example, at page 19, lines 10-16, which discloses that the term "'production'....means either expression or secretion of the protein by a mucosal cell," and that "production of a protein by the mucosal cell may be due to increased transcription." The amendment reciting "glucose-dependent insulinotropic polypeptide (GIP) promoter" is supported, for example, by claim 44, as originally filed. The amendment reciting "chromogranin A" promoter is supported, for example, at page 15, Table 1. The amendment reciting "gut mucosal tissue endocrine cells, or gastrointestinal mucosal tissue endocrine cells" was made in response to the rejection under 35 U.S.C. 112, second paragraph and, therefore, was made in order to address an informality. The amendment to claim 40 to recite "the transformed mucosal" cells was made to provide antecedent basis for this language, which is recited in amended claim 31. The amendment to claims 47, 49, 78 and 80 reciting "mucosal tissue endocrine cell or mucosal tissue stem cell, pluripotent or multipotent progenitor cell" was made to provide antecedent basis for this language, which is recited in amended claims 31 and 71. The amendment to claims 87 and 88 reciting "said transforming *in vivo*" was also made in order to provide antecedent basis for this language, which is recited in amended claims 31 and 71. Thus, as the claim amendments are supported by the specification or were made to address informalities, no new matter has been added and, entry of the amendments is respectfully requested.

Regarding the New Claims

New claims 114 to 120, which ultimately depend from either of claims 31 and 71, are supported throughout the specification. For example, claims 114 and 115 are supported, for example, at page 25, lines 24-28; and at page 42, lines 25-27. Claims 116 and 117 are supported, for example, at page 16, lines 4-6. Claims 118 and 119 are supported, for example, at page 25, lines 25-28. Claim 120 is supported, for example, at page 33, lines 10-12; at page 35, lines 26-30; at page 36, lines 23-26; and at page 27, lines 2-6. Thus, as claims 114 to 120 are supported by the specification, no new matter has been added and entry thereof is respectfully requested.

I. REJECTION UNDER 35 U.S.C. §112, SECOND PARAGRAPH

The rejection of claims 31, 34 to 36, 38, 40, 43, 47 to 49, 51, 52, 54, 55, 71 to 73, 76, 78 to 80, 82, 83 and 85 to 88 under 35 U.S.C. §112, second paragraph, as indefinite, is respectfully traversed. Allegedly, certain terms lack clarity." [Office Action, page 4]

Claims 31 and 71 are clear and definite prior to entry of the amendments set forth above. Nevertheless, solely in order to further prosecution of the application and without acquiescing to the propriety of the rejection, claims 48 and 79 have been cancelled herein without prejudice and, therefore, the rejection as to these claims is moot, and claims 31 and 71 have been amended to recite "gut mucosal tissue endocrine cells" and "gastrointestinal mucosal tissue endocrine cells." The amendment was made to address an informality and not for reasons related to patentability. In view of the amendment, claims 31, 34 to 36, 38, 40, 43, 47, 49, 51, 54, 55, 71 to 73, 76, 78, 80, 82 and 85 to 88 are clear and definite. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, second paragraph, be withdrawn.

II. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH, WRITTEN DESCRIPTION

The rejection of claims 31, 34 to 36, 38, 40, 43, 47 to 49, 51, 52, 54, 55, 71 to 73, 76, 78 to 80, 82, 83, 85 and 86 under 35 U.S.C. §112, first paragraph, as allegedly lacking an adequate written description, is respectfully traversed. The grounds for rejection are as set forth in the Office Action, pages 5-11.

The specification provides an adequate written description of claims 31, 34 to 36, 38, 40, 43, 47 to 49, 51, 52, 54, 55, 71 to 73, 76, 78 to 80, 82, 83, 85 and 86. Nevertheless, solely in

order to further prosecution of the application and without acquiescing to the propriety of the rejection, claims 48, 52, 79 and 89 to 113 have been cancelled herein without prejudice and, therefore, the rejection as to these claims is moot, and claims 1, 34 to 36, 38, 40, 43, 47, 49, 51, 54, 55, 71 to 73, 76, 78, 80, 82, 85 and 86 have been amended as set forth herein. The grounds for rejection will therefore be addressed with respect to the amended claims.

Applicants first respectfully direct the Patent Office's attention to *Falkner v. Inglis* (05-1324, Fed. Cir. 2006). In *Falkner*, the court held "that (1) examples are not necessary to support adequacy of a written description (2) the written description standard may be met (as it is here) even where actual reduction to practice of an invention is absent; and (3) there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of known structure." Thus, contrary to the statement in the Office Action, at page 7, satisfying the written description requirement does not require "a reduction to drawings or structural or chemical formulae of sufficient detail to show possession." Here, in view of the guidance in the specification and knowledge in the art, the skilled artisan would have known various functional chromagranin A promoter subsequences and variants, and sugars, polypeptides, amino acids and fats that induce transcription or secretion of proteins in mucosal tissue endocrine cells.

Functional Chromagranin A Promoter Variants and Subsequences

In terms of functional Chromagranin A promoter variants and subsequences, the specification discloses a representative sequence (Figure 14). Applicants have previously pointed out regions that have been shown or can be inferred to contribute to promoter activity (see Response filed February 10, 2005, page 14)

Applicants further respectfully direct the Examiner's attention to the Declaration under 37 C.F.R. §1.132, and accompanying Exhibit A submitted herewith. Exhibit A is a diagram summarizing knowledge of chromagranin A promoter structure and function at the time of the invention. In brief, as set forth in the Declaration (paragraph 9), the sequence region from –4800 to –2200 relative to the coding sequence has been reported to contain a positive domain, and a negative domain between –258 and –181 (see, Wu et al., J. Clin. Invest. 94:118 (1994)). The sequence region between –726 and –455 contains a 27 base pair AP-1 binding sequence (-576 to –550) that has been reported to enhance promoter activity about 10-fold (see, Nolan et al.,

Endocrinol. 136:5632 (1995); and Nolan et al., Mol. Cell. Endocrinol. 124:51 (1996)). A CRE enhancer has been reported at –147 to –100 (see, Wu et al., J. Clin. Invest. 96:568 (1995)). An Sp-1/Egr-1 site spans –88 to –77, and a cyclic AMP response element (CREB) spans 71 to –64, which have been reported to mediate gastrin activation of the promoter (see, Wu et al., J. Clin. Invest. 96:568 (1995)). A "TATA" box is present from -22 to –18, and a glucocorticoid response element (GRE) located between –583 and –597 of chromagranin A promoter was reported to confer glucocortocid responsiveness (Rozansky et al., J. Clin. Invest. 94:2357 (1994)). Most of these Chromagranin A promoter regions have been reported or are believed to be functionally conserved between rat, mouse and human. Thus, in view of the foregoing, the skilled artisan would have knowledge of Chromagranin A promoter structure and function.

As also set forth in the Declaration, Chromagranin A promoter sequences exhibit significant inter-species sequence homology (paragraph 9). For example, mouse and rat chromagranin A promoter share 85% homology and mouse chromagranin A promoter shares 52% homology with the bovine promoter. Two regions of mouse and human chromagranin A promoter, from -1107 to -1040 and from -5752 to -5475 relative to the putative transcription start site, share 78% and 85% identity, respectively.

Based upon knowledge of chromagranin A promoter structure and function, chromagranin A promoter functional conservation and sequence homology between species at the time the application was filed, Dr. Cheung has concluded that functional chromagranin A promoter subsequences and variants would be known to the skilled artisan (paragraph 10).

In sum, in view of knowledge of chromagranin A promoter structure and function, chromagranin A promoter functional conservation and species sequence homology, the skilled artisan would have possessed knowledge of various functional chromagranin A promoter subsequences and variants at the time of the invention. Consequently, an adequate written description is provided for functional chromagranin A promoter subsequences and variants and, Applicants respectfully request withdrawal of this ground for rejection under 35 U.S.C. §112, first paragraph.

Sugars, Polypeptides, Amino Acids and Fats

The claims have been amended as set forth above to recite that a sugar, polypeptide, amino acid or fat induces "transcription or secretion." As set forth in the record, structural

features that characterize sugars, polypeptides, amino acids and fats were known to the skilled artisan at the time of the invention (see, Response to Office Action filed October 7, 2005, pages 10-12).

In terms of sugars, polypeptides, amino acids and fats that induce "transcription" of insulin or leptin by transformed mucosal tissue endocrine cells, the claims recite "GIP promoter" or "chromogranin A promoter." As disclosed in the specification and acknowledged by the Examiner, glucose can increase GIP promoter activity thereby inducing transcription of an operably linked gene (see, page 13, lines 21-26; and page 14, lines 10-12). With respect to chromogranin A promoter, activity of this promoter increases in response to gastrin (see, Response to Office Action filed February 10, 2005, page 14). As disclosed in the specification, amino acids induce gastrin secretion by G-cells (page 27, Table 2). Consequently, amino acids (e.g., from digested polypeptides) can increase chromogranin A promoter activity, via gastrin produced by G-cells, thereby inducing transcription of an operably linked gene. Thus, in view of the foregoing, the skilled artisan would know that amino acids and polypeptides (degraded into amino acids) can increase chromogranin A promoter activity thereby inducing transcription and production of proteins in transformed gut and gastrointestinal mucosal tissue endocrine cells.

In terms of sugars, polypeptides, amino acids and fats that induce "secretion" of proteins by mucosal tissue endocrine cells, gut and gastrointestinal mucosal tissue endocrine cells are naturally able to secrete various proteins in response to various nutrients including, for example, sugars, polypeptides, amino acids and fats (see, for example, page 25, lines 6-11). In this regard, the specification discloses data demonstrating that mice in which mucosal tissue endocrine cells were transformed with a sequence encoding human insulin rapidly produced human insulin in response to oral glucose or a mixed meal (page 48, lines 16-21, Figure 11B), and that glucose is rapidly cleared from STZ-treated mice (Figure 12). In addition to the foregoing data, human C-peptide, a by product of insulin, is produced within 15 minutes of oral glucose administration to animals transformed with a GIP-insulin bearing vector (see the Declaration under 37 C.F.R. §1.132 executed by Dr. Anthony Cheung, paragraph 11, *Results*, and accompanying Exhibit B, panel C, submitted herewith. The kinetics of rapid insulin production and glucose clearance indicate that glucose and a mixed meal induce insulin secretion. Thus, in view of the specification (corroborated by the data in Exhibit B, panel C), the skilled artisan would know

that glucose and mixed meals can induce secretion of proteins by gut and gastrointestinal mucosal tissue endocrine cells.

The specification discloses other nutrients ("secretagogues") that induce secretion of proteins by gut or gastrointestinal endocrine cells (page 27, Table 2). To name a few, for example, amino acids induce gastrin secretion by G-cells; amino acids induce cholecystokinin secretion by I-cells, and glucose and fat induce secretion of glucagon-like peptide 1 and peptide 2 from L-cells. Thus, in view of the guidance in the specification, the skilled artisan would have known that glucose, a mixed meal, amino acids and fats, also can induce secretion of proteins by gut and gastrointestinal mucosal tissue endocrine cells.

As further support of the position that the skilled artisan would know sugars, polypeptides, amino acids and fats that can induce secretion of proteins by mucosal tissue endocrine cells, Applicants cited 15 corroborating references in the Response filed October 7, 2005. Of the 15 references, Applicants submit copies of three representative references, Exhibits E to G, Flatt *et al.*, J. Nutr. 119:1300 (1989); Schulz *et al.*, Scand. J. Gastroenterol. 17:357 (1982); and Falko *et al.*, J. Clin. Endocrinol. Metab. 41:260 (1975), respectively. Each of Exhibits E to G demonstrate that GIP, produced by K cells (see specification, page 27, Table 2), is secreted in response to sugars, amino acids and fats. In Exhibit E, the authors report that oral administration of sugars and sugar analogues (glucose, galactose, fructose, 3-O-methylglucose and alpha-methyl-glucoside) to ob/ob mice stimulated prompt (within 30 minutes) release of GIP. In Exhibit F, the authors report increased GIP release in response to duodenal instillation of glucose and amino acids. In Exhibit G, the authors report that fat is a potent stimulus for release of GIP in normal individuals. Thus, in view of the foregoing, the skilled artisan would have known various sugars, amino acids and fats that can induce secretion of proteins by mucosal tissue endocrine cells.

In sum, in view of the foregoing guidance in the specification and knowledge in the art the skilled artisan would have possessed knowledge of sugars, polypeptides, amino acids and fats that induce transcription or secretion of proteins by mucosal tissue endocrine cells. Consequently, an adequate written description is provided for sugars, polypeptides, amino acids and fats and, Applicants respectfully request withdrawal of this ground for rejection under 35 U.S.C. §112, first paragraph.

III. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH, ENABLEMENT

The rejection of claims 31, 34 to 36, 38, 40, 43, 47 to 49, 51, 52, 54, 55, 71 to 73,76, 78 to 80, 82, 83, 85 to 88 under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement is respectfully traversed. The claims have been rejected for grounds of record as well as new grounds set forth in the Office Action, pages 12 to 33.

The claims are adequately enabled prior to entry of the Response, for the reasons of record. Nevertheless, without acquiescing to the propriety of the rejection and solely in order to further prosecution of the application, claims 48, 52, 79, 83 and 89 to 113 have been cancelled herein without prejudice rendering the rejection moot. Claims 31 and 71 have been amended to delete "at risk of," and to recite that "said transforming occurs *in vivo* via intra-cavity delivery to stomach or small intestine, thereby producing transformed....cells," and "wherein orally contacting" said transformed cells with an amount of sugar, polypeptide, amino acid or fat induces "transcription or secretion" of the insulin or leptin. The rejection will therefore be addressed insofar as it may pertain to claims 31, 34 to 36, 38, 40, 43, 47, 49, 51, 54, 55, 71 to 73, 76, 78, 80, 82, 85 to 88 and 114 to 120 upon entry of the Response.

In Vivo Transformation and Vectors

As set forth in the record, Applicants have provided substantial evidence corroborating that cells can be transformed *in vivo* with different viral vectors, different genes and different promoters, by intra-cavity delivery, and that transformed cells can produce functional and therapeutically relevant levels of proteins *in vivo*, as claimed. Applicants submit herewith additional evidence corroborating that cells can be transformed *in vivo* with non-viral vectors, by intra-cavity delivery, and that transformed cells can produce functional and therapeutically relevant levels of protein *in vivo*, as claimed.

Evidence of Record:

1) Previously submitted Exhibits 1 and 2, a sworn Declaration under 37 C.F.R. §1.132 and accompanying Figures 1-4, respectively, filed June 16, 2004, included data demonstrating *in vivo* transformation into gut or gastrointestinal mucosal tissue of animals with three different genes (insulin, DsRed and GFP) using two different vectors, FIV and AAV2. Mucosal cells transformed *in vivo* with human insulin gene in FIV vector produced human insulin at therapeutically relevant levels sufficient to reduce glucose, and over a long term. Two other

genes, DsRed and GFP, were present in mucosal tissue of animals following *in vivo* mucosal cell transformation with AAV2 and FIV vectors. Copy numbers of the transformed genes after 14 days were comparable between AAV2 and FIV, indicating that AAV2 vectors will confer expression of proteins at therapeutically relevant levels. The data in previously submitted Exhibits 1 and 2 therefore corroborates that gene transfer into gut or gastrointestinal mucosal tissue *in vivo* is A) independent of the particular gene transferred; B) can be performed with different vectors; and C) is sufficient to confer expression of proteins at therapeutically relevant levels.

2) Previously submitted Exhibit AA, a sworn Declaration under 37 C.F.R. §1.132 and accompanying Figures 1 and 2, filed February 10, 2005, included data and results of *in vivo* transformation with insulin and SEAP. In brief, gut or gastrointestinal mucosal cells were transformed *in vivo* by injection of an FIV vector bearing an insulin or SEAP gene, driven by a GIP or chromagranin A promoter, into gut lumen. Transformed cells in the animals produced 1) insulin and SEAP at therapeutically relevant levels long term; and 2) insulin 150 days after delivery allowing animals to survive STZ- treatment, demonstrating that insulin was functional and therapeutic.

The foregoing data of record therefore corroborates that gut or gastrointestinal mucosal tissue of animals an be transformed with genes driven by different promoters *in vivo* via intra-cavity delivery, and the transformed cells can A) express functional proteins at therapeutic levels; and B) express proteins for long periods of time.

Newly Submitted Evidence: Transformation with Non-viral and Viral Vectors In Vivo and Production of Therapeutic Levels of Insulin in Animals

Studies corroborating that *in vivo* transformation can be performed with other vectors, including non-viral vectors, and that the transformed gut or gastronintestinal mucosal cells produce proteins at therapeutic levels in animals were performed as described in paragraph 11 of the 132 Declaration. In brief, chitosan-DNA nanoparticles, an expression plasmid for ϕC31 integrase and a targeting plasmid containing the GIP promoter linked human insulin gene, were produced as described. FIV and six AAV vectors (AAV1, AAV2, AAV2.5, AAV5, AAV6 and AAV8) were also produced, each of which contained a marker gene driven by a rat GIP

(paragraph 11). Chitosan-DNA nanoparticles and viral vectors were delivered into duodenum of male mice (C57/BL6) by a single injection.

Gene copy numbers were measured by quantitative PCR of duodenal tissue samples two weeks after vector delivery. Transformed genes were present in mucosal tissue after 14 days (paragraph 11, *Results* and Exhibit B, panel A), indicating that the vector integrated the insulin gene into a stem or precursor cell population, consistent with previous data (see, for example, Exhibit 2, Figures 1 and 2, submitted June 18, 2004). Gene copy numbers were highest with non-viral chitosan-DNA nanoparticles. Six of seven viral vectors (FIV, AAV1, AAV2, AAV2.5, AAV5 and AAV6) achieved somewhat less copy numbers but all had levels comparable to FIV (paragraph 11, *Results* and Exhibit B, panel A). In this regard, numbers of gene copies achieved with FIV vector were previously shown to be sufficient for insulin production at therapeutic levels (see, for example, Exhibit 2, Figures 2A and 3, submitted June 18, 2004). Because five of the six AAV vectors achieved gene copy numbers comparable to those of FIV vector, these AAV vectors are predicted to also confer insulin production at therapeutic levels in animals.

Human C-peptide levels were measured in blood samples using antibodies that specifically recognize human C-peptide. As shown in Exhibit B, panel B, a single administration of chitosan-packaged plasmids resulted in continuous production of human C-peptide for over 115 days (paragraph 11, *Results*). Furthermore, secretion of human C-peptide was inducible by oral glucose administration to the animals (Exhibit B, panel C), indicating that the human insulin transgene was targeted to appropriate cells. These results indicate that a single administration of chitosan encapsulated plasmid DNA, containing the GIP promoter-linked human insulin gene, to lumen of mice duodenum, resulted in systemic long–term production of orally glucose-inducible human insulin at therapeutic levels.

Based upon the foregoing results and the data of record, Dr. Cheung has concluded that viral and non-viral vectors can be used to deliver insulin or leptin into gut or gastrointestinal cells *in vivo* to produce transformed cells without undue experimentation (paragraph 12). Dr. Cheung has also concluded that 1) transformed cells can exhibit long term production of the encoded proteins; 2) insulin or leptin is produced at levels sufficient to treat animals, indicating that therapeutic levels can be produced; and 3) insulin and leptin can be produced in response to glucose and other nutrients (paragraph 12).

Cells other than K cells Produce Insulin

With respect to cells other than K cells producing insulin, Applicants respectfully direct the Examiner's attention to the 132 Declaration submitted herewith, which includes a description of studies and data indicating that gut or gastrointestinal mucosal cells other than K cells produce insulin (paragraph 13, and Exhibit C). In brief, STC-1 cells, a mixed population of entero-endocrine cells that include S-cells (produce secretin); L-cells (produce proglucagon related peptides); K cells (produce GIP); D-cells (produce somatostatin); cholecystokinin producing cells; and neurotensiin producing cells, were transfected with a plasmid carrying a CMV promoter-driven human insulin construct and analyzed for insulin and GIP production (paragraph 13). As expected, bioactive human insulin was detected in K-cells that express GIP (Exhibit C, panel A). In addition, non-K gut endocrine cells within the transfected STC-1 cells (non K cells do not stain for GIP) produced bioactive insulin (Exhibit C, panel B).

Dr. Cheung has concluded that in view of the foregoing studies, that gut or gastrointestinal endocrine cells other than K-cells can process proinsulin into bioactive insulin (paragraph 14). Dr. Cheung has predicted that because endocrine cells other than K-cells can produce bioactive insulin, that these other endocrine cells will secrete bioactive insulin in response to a sugar, polypeptide, amino acid or fat (paragraph 14).

In terms of the cited Yoon et al. publication (Trends Mol. Med. 8:62 (2002)), and the types of cells that can produce insulin, the specification discloses various cell types that produce various proteins in response to various nutrients (see Table 2, page 27). Such cell types include, G-cells, D-cells, K-cells, L-cells, I-cells, Mo-cells and Gr-cells. K cells are one of the cell types shown to produce insulin, and both GIP and chromagranin A promoters are active in K cells. In addition, the foregoing data submitted herewith and discussed above corroborates that gut or gastrointestinal endocrine cells other than K-cells can process proinsulin into bioactive insulin (Exhibit C, panel B). Thus, endocrine cells other than K-cells can produce insulin in response to a sugar, polypeptide, amino acid or fat, as claimed.

Nutrients other than Glucose

As discussed above, the activity of chromogranin A promoter increases in response to gastrin and, amino acids induce gastrin secretion by G-cells. Consequently, amino acids can

increase chromogranin A promoter activity, via gastrin produced by G-cells, thereby inducing transcription of a gene operably linked to chromogranin A promoter. Thus, amino acids and polypeptides can induce transcription of proteins in gut and gastrointestinal mucosal tissue endocrine cells.

As also discussed above, gut and gastrointestinal mucosal tissue endocrine cells are naturally able to secrete protein in response to various nutrients including, for example, sugars, polypeptides, amino acids and fats. In particular, for example, the specification discloses nutrients ("secretagogues") that induce secretion of proteins by gut or gastrointestinal endocrine cells (page 27, Table 2). For example, amino acids induce gastrin secretion by G-cells; amino acids induce cholecystokinin secretion by I-cells, and glucose and fat induce secretion of glucagon-like peptide 1 and peptide 2 from L-cells. Thus, in view of the foregoing, a mixed meal, amino acids and fats can induce secretion of proteins by gut and gastrointestinal mucosal tissue endocrine cells.

As further discussed above, Applicants cited 15 corroborating references in the Response filed October 7, 2005, and copies of three representative references, Exhibits E to G, are submitted herewith. Each of Exhibits E to G report that GIP, produced by K cells is produced in response to various sugars, amino acids and fats. Thus, various sugars, amino acids and fats can induce secretion of proteins by mucosal tissue endocrine cells.

Leptin Treatment

With respect to subject's amenable to leptin treatment according to claims 71 and claims depending therefrom, Applicants submit that in view of the guidance in the specification and knowledge in the art, the skilled artisan could reasonably predict candidate subjects for leptin treatment. First, the specification discloses candidate subjects for leptin treatment, including obese subjects or those having an undesirable body mass (page 33, lines 21-25). Subjects include those having a disorder or at risk of developing the disorder (page 33, line 29, to page 34, line 1. The specification discloses at risk subjects including those whose diet may contribute to or be associated with obesity, as well as those with a family history or genetic predisposition towards development of obesity (page 34, lines 1-5). Thus, in view of the guidance in the specification, the skilled artisan would know that subjects amenable to leptin treatment include obese subjects, those having an undesirable body mass, and subjects whose diet or genetic

predisposition towards obesity, e.g., a subject with a leptin deficiency. Consequently, the specification discloses candidate subjects for leptin treatment.

Consistent with the teachings in the specification, previously submitted Exhibit A, a sworn Declaration under 37 C.F.R. §1.132 and accompanying Figures 1-11, filed June 18, 2003, included animal studies in which leptin expressed by gut cells transformed *in vitro*, when implanted into animals (ob/ob mice), was effective to cause weight loss and normalize glucose levels in the animals. Accordingly, in view of the guidance in the specification and corroborating data in previously submitted Exhibit A, it is clear that a leptin deficient mammalian subject can reasonably be predicted to respond to leptin treatment.

In addition to a leptin deficient mammalian subjects, as previously pointed out in the Response to Office Action filed October 7, 2005, previously submitted Exhibit A, a publication by Heymsfield et al. (JAMA 282:1568 (1999)), corroborates that humans that express leptin can be treated in accordance with the invention methods. In brief, Heymsfield et al. reported that weight loss occurred in both lean and obese humans administered leptin (see results, page 1568). Furthermore, the authors reported that leptin appeared to induce weight loss in obese humans *with elevated endogenous serum leptin levels* (see Conclusions, page 1568, *Emphasis added*). Consequently, in view of Heymsfield et al., a mammalian subject that expresses leptin, including mammalian subjects with elevated leptin, are candidates for treatment in accordance with the invention methods.

In addition to the foregoing mammalian subjects, Exhibit D submitted herewith (Orci et al., Proc. Natl. Acad. Sci. USA 101:2058 (2004)) includes studies of Zucker rats, which have a mutant leptin receptor that results in obesity. The authors report that administration of an adenovirus vector bearing leptin cDNA driven by a CMV promoter reduced body weight and increased plasma leptin levels in treated Zucker rats (see 132 Declaration, paragraph 15, and Exhibit D, page 2058, Experimental Procedures, and page 2059, Results). Consequently, in view of Exhibit D, Dr. Cheung has concluded that a mammalian subject resistant to leptin is a candidate for treatment in accordance with the claimed methods (paragraph 16).

In sum, in view of the guidance in the specification, and corroborating data and reports by other investigators indicating that a variety of mammalian subjects can respond to leptin treatment, namely 1) subjects with inadequate or deficient amounts of leptin; 2) subjects that produce leptin; 3) subjects that overproduce leptin; and 4) subjects resistant to leptin, the skilled

artisan could reasonably predict mammalian subject's for leptin treatment in the claimed methods.

In view of the foregoing guidance in the specification, corroborated by the evidence of record and as submitted herewith and supporting publications of record and as submitted herewith, the skilled artisan could practice claims 31, 34 to 36, 38, 40, 43, 47, 49, 51, 54, 55, 71 to 73, 76, 78, 80, 82, 85 to 88 and 114 to 120 without undue experimentation. As such, the claims are adequately enabled and the rejection under 35 U.S.C. §112, first paragraph, is improper and must be withdrawn.

CONCLUSION

In summary, for the reasons set forth herein, Applicants maintain that claims 31, 34 to 36, 38, 40, 43, 47, 49, 51, 54, 55, 71 to 73, 76, 78, 80, 82, 85 to 88 and 114 to 120 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 509-4065.

Please charge any fees associated with the submission of this paper to Deposit Account Number 502212. The Commissioner for Patents is also authorized to credit any over payments to the above-referenced Deposit Account.

Respectfully submitted,

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CERTIFICATION UNDER 37 C.F.R. §§ 1.8 and/or 1.10*

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